

United States Department of Agriculture
Grain Inspection, Packers and Stockyards Administration
Federal Grain Inspection Service

FGIS Issuance Change

CHANGE TO☐ DIRECTIVE☐ MANUAL☒ HANDBOOK

CHANGE NO:
6**TO (No.)****TITLE:**
DON (Vomitoxin) Handbook**DATE:**
3-12-07

PURPOSE OF CHANGE: The DON (Vomitoxin) Handbook, Chapter 13, Charm Science Rosa® P/N Test kit, page 13-1, has been revised to show a change in the preparation instructions for the Positive Controls.

FILING INSTRUCTIONS

Remove	Dated	Insert	Dated
Chapter 13, Page 13-1	9-11-06	Chapter 13, Page 13-1	3-12-07
Chapter 13, Page 13-2	No Date	Chapter 13, Page 13-2	No Date

Retain this issuance sheet as an aid in verifying handbook contents.

/s/ John Giler

John Giler, Acting Director
Field Management Division

Distribution: A, C, E

Originating Office: PPB, FMD

13.1 GENERAL INFORMATION

The ROSA® DON P/N test kit uses lateral flow test strip technology that provides qualitative (equal to or less than a specified threshold) results in wheat and barley. Screening levels of 0.5 ppm (wheat only) and 1.0 ppm (wheat or barley) both available in this kit based on the extract volume used in test.

13.2 PREPARATION OF TESTING MATERIALS

NOTE: A Negative and Positive Control should be run periodically using the Performance Monitoring Mode (see section 13.4 e.) to verify performance of equipment and test strips (daily, weekly, bi-weekly, or monthly, based on internal quality assurance standards).

a. Negative Control.

Use the DON Dilution Buffer for the Negative Control.

b. Positive Control.

Prepare the 0.5 ppm DON Positive Control by adding 6.0 ml of DON Dilution Buffer and mix for 30 seconds. Allow to stand for 10 minutes at room temperature. Mix again before using. Use 300 µl as your diluted extract and test following Test Procedures section 13.4 (c). **A valid positive test result must be received before official testing.**

NOTE: Store at 32-45 °F for up to one week, or freeze at -4 °F for 2 months.

c. Equipment Preparation.

(1) Incubator must be at $45 \pm 1^{\circ}\text{C}$ (temperature indicator is green).

(2) Incubator must be clean and level.

d. DON Dilution Buffer.

(1) Predispense 1.0 ml of DON Dilution Buffer into a micro-centrifuge tube for each sample to be tested.

(2) Use this solution at room temperature.

(3) Store DON Dilution bottle and any unused predispensed tubes at 32-45 °F.

e. Test Strips.

- (1) Remove ROSA® DON P/N moisture resistant container from the refrigerator and allow it to reach room temperature to limit condensation.
- (2) Remove only the number of strips to be used and return container to 32-45 °F storage. Strips are stable at room temperature for at least 12 hours.

NOTE: If blue desiccant packets turn white or pink, performance test the strips with Negative and Positive Controls before continued use.

13.3 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into a clean extraction container.
- b. Add 250 ml of the deionized or distilled water.
- c. Blend or shake for a minimum of 60 seconds. Allow sample to settle for 1 minute to obtain sample extract.

NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. **To Filter:** pour the extract into Whatman 2V (or equivalent) filter paper and filter into a labeled collection container. **To Centrifuge:** transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. Clarified extract is now ready for testing.

13.4 TEST PROCEDURES

a. Sample Preparation for 0.5 ppm Screening Level

- (1) Pipet 100 µl of clarified extract to a predispensed (1.0 ml DON Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract.
- (2) Label the test strip to identify sample.
- (3) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.